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ABSTRACT

Troops deployed in the Persian Gulf War were exposed to an unusually diverse mix of odorous chemicals at the same time as they were exposed to physiological and psychological stressors B a scenario that research in animal models suggests will lead to the development of specific conditioned responses. The goal of this research is to investigate the extent to which people can acquire stress reactions as conditioned responses to odors and exhibit health symptoms as a result of such conditioning episodes. Thus, the paradigm investigated in this project can serve as a model system for examining and understanding the persistent symptom constellations found in GWS and other stress-mediated syndromes. Results from the first three studies strongly suggest that odor-stress conditioning can powerfully mediate elevations in hormonal status (salivary cortisol) self-reported stress, health symptoms and judged cognitive effort on memory tests, and that cognitive information about the nature of the chemical odor may enhance the stress and health symptom reports over that which is due to conditioning alone. Current studies are continuing to explore additional parameters of the odor-stress conditioning paradigm.

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INTRODUCTION:

The overall goal of this project is to investigate the hypothesis that the symptom constellation of Gulf War Syndrome (GWS) and other stress-mediated illnesses stemming from military deployment can be understood as conditioned responses to chemical odors encountered under stressful conditions (Bouton, Barlow & Mineka 2001). The specific goals of Year 4 were conduct two studies to examine (1) the degree to which a conditioned response could be inhibited by prior exposure to the odor in the absence of the stressor stimulus and (2) to evaluate whether the conditioned response to the odor could be blocked by pre-associating it with another odorant. We also attempted to complete the study exploring the degree to which an ecologically valid stressor (hazard training video) can produce heightened conditioned stress response to odors among professional emergency responders (Study 4); however, difficulties in recruiting and scheduling this study population, as well as the need to complete the two other studies during this time period have delayed the completion of this study. As commencement of the research was delayed one full year at the start due to concerns raised by the Army HUC, our ability to conduct this important ecologically relevant study has been delayed. Thus, we have requested and have been granted a one-year, no-cost extension for the purpose of finishing this study.

BODY:

Study 7, entitled "Latent Inhibition and Prevention of Odor-Stress Associations", investigated whether pre-exposure to an odor stimulus would retard the ability of that odor to become associated with a stressor and reduce the occurrence of a conditioned odor-stress response. 48 volunteers had two sessions of pre-exposure to an odorant paired with a relaxation and a stressful task in counter-balanced order; following that they had one session of exposure to the odor alone and then were tested to observe whether the odor-stress conditioning occurred as measured by evaluation of autonomic arousal, cognitive function and self-reported stress and health symptoms. Data collection has been completed for this study and data analysis is largely complete.

Study 8, entitled "Blocking and Prevention of Odor-Stress Associations", investigated the extent to which pre-associating a novel "scapegoat" odor with a stressor could neutralize the subsequent ability of other odors to become associated with stressors and prevent the acquisition

of conditioned odor-stress responses. 36 subjects (out of a total of 40) have been tested in three sessions in which they were exposed to either novel, unusual odors or more familiar ones, paired with stressors; the degree of odor-stress conditioning that occurred was measured by evaluation of autonomic arousal, cognitive function and self-reported stress and health symptoms.

Testing of human research participants in the studies listed in the approved SOW for Year 3 continued with Study 4. However, due to multiple scheduling constraints for the planned subject population, this study has not yet been completed, but is still ongoing. While data on the first 17 subjects are presented, we have requested (and have been granted) a no-cost extension in order to complete this study.

General Subject Recruitment and Screening

Participants are recruited using flyers, advertisements placed in local newspapers or selected from our subject database. For recruitment in Study 4, we have approached local professional associations for emergency responders and firefighters in order to more easily make contact with interested individuals in these groups. Individuals who express an interest in participating in our studies are invited for an information and screening session in which they are provided with information about the nature of this study. By signing a special (independent of this study) consent form, they give us permission to collect and store relevant demographic data in our database for the purpose of screening for eligibility for our studies. During the first session, they complete a self-report medical/occupational history and Chemical Intolerance Index (Lees, Stefaniak, Emmett, & Dalton, 2003). They are also tested for their olfactory abilities on a 7-item olfactory discrimination task, to ensure their ability to detect and process the experimental odors (Dalton, Gould, Girten, Stodieck, & Bateman, 2003). For the screening session, they receive financial remuneration of $10. We rely on self-report history with confidence, because it has been our experience that our procedures pose only very minor, if any, risk of physical harm or lasting psychological distress.

Individuals (m/f) between ages 18-55, in good general health, with average olfactory abilities, no occupational history of chemical exposure, no chemical sensitivity, medical diagnosis of cardiovascular disease, or asthma, no pacemaker, and no psychiatric diagnoses of Chronic Fatigue Syndrome, Posttraumatic Stress Syndrome, Depression, Anxiety Disorders, Burnout Syndrome or Claustrophobia are eligible for our experiments. We regularly test an

ethnically diverse group composed of roughly equal numbers of males and females (see enrollment tables for each study). However, in order to comply with the experimental instructions, all participants have to be able to speak and understand English well.

Exclusion criteria:

Demographics: criteria related to demographics are collected using the medical/occupational history screening form. Individuals younger than 18 years old and older than 55 years and who are non-English speakers are excluded from our studies.

Chemical Intolerance: Participants who report regular to frequent sickness from chemical, synthetic odors (a score of 3 or above across the board, or some 4's and 5's for chemical odors) are excluded. To date, we have only had to reject three subjects based on these criteria.

Sense of smell: Participants who indicate a sense of smell much worse than most people's, or do not pass the criterion on the 7-term odor identification task are excluded from the study.

Medical criteria: Participants who answered "yes" to any of the following medical conditions will be excluded from the studies: asthma, severe seasonal or perennial allergies, chronic sinusitis, deviated septum, a head injury with loss of conscience, cardiovascular (heart) disease, high blood pressure, or if they have a pacemaker.

Exposure history criteria: Individuals who indicated a prolonged (> 1 year) occupational exposure history to pesticides, industrial solvents or formaldehyde are excluded from participation.

Psychiatric criteria: Individuals who indicated to have or have had any of the following conditions are excluded from participation: Chronic Fatigue Syndrome, Posttraumatic Stress Syndrome, Depression, Anxiety Disorders, Burnout Syndrome or Claustrophobia.

SOW FOR YEAR 2 (delayed, to be completed in Year 5 – no –cost extension)

Study 4, entitled "Association of An Odor (CS) To Multiple Real-World Stress Stimuli" will investigate the feasibility of a stress induction procedure other than the Trier Social Stress Test for use in the laboratory. Police Academy trainee personnel will watch emergency responder training videos, which are expected to engage their belief-system and, consequently, cause stress

and arousal as a US. This stressor is presumed to have more ecological validity than the Trier Test. Study 4 will follow the design of Study 1 as described below; 48 subjects will be tested in two sessions. Measures of autonomic arousal, cognitive function and self-reported stress and health symptoms will be collected.

Measures of Stress Response: In this study, as well as all others conducted in this project, we have sought to obtain multiple converging measures of a clinically significant stress response, including self-reported anxiety, cortisol levels (a widely-used measure of stress response), psychophysiological parameters of arousal (including heart rate and electrodermal activity), perceived health symptoms and performance disruption on a standardized memory test (the California Verbal Learning Test). While none of these measures by themselves may be indicative of clinically significant levels of stress, we are aiming to develop a profile that may be predictive of a stress response in a real-world situation. Thus, some of the measures are largely exploratory ones, while others (self-reported stress and cortisol response, memory disruption) have considerable validity in the realm of stress research.

Initial responses suggest a wide variability in autonomic responses to the stressors within this cohort. However, the small sample size (n=5/gp) precluded any formal analysis to determine significance. We do intend to correlate changes in autonomic response to the stressor with personality subtypes (e.g., Negative Affectivity) in order to partition out some of the variation in response, if it is due to differences in personality traits.

Modification to Study Design: A change in the protocol was proposed and implemented which has facilitated subject recruitment. In all prior studies, incorporating both the stressor and relaxation phase in each session caused the session to exceed 2.5 hours per day (and in some cases, it exceeded 3 hours/ session). We were able to recruit very few subjects, meeting our eligibility criteria for this study, who were able to participate for the necessary time commitment. Hence, given the importance of demonstrating a conditioned stress-response to an odor cue under more environmentally realistic conditions, we pilot tested omitting the second part of the session during which odors were paired with a relaxation induction in order to keep the session under 1.5 hours per day and allow us to collect data in a reasonable pace. This was viewed as especially important given the impact on the project timetable that occurred following the delay in obtaining HUC approval during all of Year 1. However, in order to ensure that the subject was

not stressed when leaving the session, we had them participate in the relaxation induction in a separate room, but without collecting physiological data and in the absence of any odor. Consequently, this did not require any significant modification to the description of the procedure in the consent form.

The table below represented the new design (strikeouts indicate changes from the prior design.).

Design: Table 1. New Design of Study 4

Group	Conditioning Phase	Test Phase
1 (Congruent)	CS_b + 20 min.stressor ~~CS_a + 20 min. relaxation~~	CS_a - HR/Resp/Startle/Cog. ~~CS_b - HR/Resp/Startle/Cog.~~
~~2 (Incongruent)~~	~~CS_a + 20 min.stressor~~ ~~CS_b + 20 min. relaxation~~	~~CS_a - HR/Resp/Startle/Cog.~~ ~~CS_b - HR/Resp/Startle/Cog.~~
3 (Control)	+ 20min. stressor ~~-20 min. relaxation~~	CS_a - HR/Resp/Startle/Cog. ~~CS_b - HR/Resp/Startle/Cog~~

Design: Group 1 was and will be exposed to the odor in the presence of the stressor for a period of 20 minutes. Due to the proposed elimination of the relaxation condition, there is currently no need to test a second group with an alternate odor. This elimination allowed us to add additional subjects to each of the remaining conditions (Group 1 and Group 3). A control condition, Group 3, was and will be exposed to the US (stressor) but without an odor, in order to evaluate the strength of conditioning that occurs to the context (room) alone. During the conditioning phase and the test phase, we will monitor heart rate and respiration rate of each participant, as measures of autonomic arousal; we will also evaluate subjective symptom reports and mood. The test phase will utilize these measures as well as several additional dependent measures, including a test of cognitive function (short-term and general memory performance). In both conditioning and test phases we will collect salivary samples 8 times in order to measure cortisol levels. Twenty-four subjects will be tested in each group, yielding a total of 48 subjects.

Procedure: The study is introduced to the subject as a study about the influence of odors on cognitive performance and attention. The timetable and schedule of dependent measures will remain as indicated in Table 2. During Session 1 (the conditioning session), the subject will fill

out personality questionnaires for half an hour, to allow for serum cortisol levels and any anticipatory stress related to participating in a study to decrease to a comfortable baseline level. Thereupon, the subject enters the environmental chamber, where electrodes are connected to the subject's body for 10 minutes of baseline biomonitoring of autonomic endpoints. After 10 minutes have elapsed, the subject is shown a training video which portrays the outcome of a biological/chemical warfare attack and the actions to be taken by emergency personnel under such circumstances. During this session, subjects in one group are exposed to the odor while subjects in the other group do not experience an odor. Once the odor is dispersed into the room, they complete sensory ratings while various physiological endpoints are measured (see Table 3).

Measures:

Table 2: Timetable of events and endpoints

Prechamber		Chamber	Session 1: Odor a/b + Stressor Session 2: Odor a/b + Test					
	T-40	T-10	T0	T+10	T+20	T+30	T+40	T+50
Questionnaires	X							
Cortisol	Y	Y				Y		Y
VAS	Y	Y	Y	Y	Y	Y	Y	Y
Symptom		Y			Y			Y
Mood		Y			Y			Y
Intensity ratings		Y*						
HR/Resp/~~EDA~~		Y**						
Memory			Z**			Z**		

Note: The symbols X, Y, Z denote when the given measures were collected: X was measured only during Session 1, Y during both Session 1 and 2, and Z only during Session 2.

* Odor intensity ratings were collected every 5 minutes throughout the stay in the chamber

** These measures were collected continuously throughout exposure

VAS = Visual Analog Scales, HR= Heart Rate, Resp = Respiratory Rate, EDA=Electrodermal Activity,

The following endpoints were measured *at both conditioning and test sessions*:

Stress: Salivary samples for cortisol assessments were obtained upon arrival (Baseline 1: T-40), 10 minutes after entering chamber (Baseline 2:T0), 20 minutes into the performance phase of the TSST (T+20). Subjective ratings of perceived stress and anxiety were rated on Visual Analogue Scales at the same time-points when saliva samples were obtained. Saliva samples were obtained by having subjects chew on a salivette for 2 minutes, which were then expectorated into a vial. Vials were centrifuged and the resulting supernatant was transferred into a cryo-vial and kept frozen at -80 C, until transferred to the laboratory for analysis.

Odor, irritation and annoyance intensity ratings: While in the chamber, the subject rated the intensity of the odor, sensory irritation and annoyance on a computer version of the Labeled Magnitude Scale (LMS) every five minutes. The LMS is a category-ratio scale that has ratio properties while using category labels to guide the placement of the rating (Green et al., 1996). Ratings can range from no sensation to strongest imaginable.

Mood State: Current mood states were assessed, using the Profile of Mood States, just prior to entering the chamber and after the CS+ and CS-conditions (McNair, Lorr, & Droppleman, 1992).

Health symptoms: Health symptoms were rated on a laptop just prior to entering the chamber, and after the CS+ and CS-conditions. Health symptoms were grouped into seven categories: cognitive, sensory irritation, central nervous system, autonomic, respiratory, GI and sham (Smeets, Maute, & Dalton, 2002).

Autonomic arousal: Respiratory rate/volume and heart rate were continuously monitored throughout baseline, the stressor task and the relaxation phase, using the Lablinc V system (Coulbourn, Allentown, PA). Startle response in prior studies was the least informative (and most variable) of the autonomic measures. In addition, because of the stress-induced activity, perspiration was interfering with the integrity of the electrode placement and the signal contact. To ensure that the measure itself did not elicit more stress during the final test phase, it was necessary to place electrodes during each session, even though measurements were not being made until the last session. For these reasons, it was deemed advisable to eliminate this measure.

The following endpoints were measured *only during the test phase*:

Cognitive Function: To evaluate the degree to which conditioned stress can impair cognitive function, we administered the California Verbal Learning Test (CVLT) as a measure of learning and memory compared with the subject's own assessment of their performance on these tests. The CVLT was administered only during the test session. Because subjects' evaluations of their performance on neuropsychological tasks have been found to be more closely related to affective distress than to actual performance (Binder et al., 1999), subjects also rated their performance on a 10 point Likert Scale.

TAKEN FROM THE SOW FOR YEAR 4

Study 7, entitled "Latent Inhibition and Prevention of Odor-Stress Associations", investigated whether pre-exposure to an odor stimulus would retard the ability of that odor to become associated with a stressor and later elicit a conditioned odor-stress response. 48 volunteers were given two sessions of pre-exposure to an odorant paired with a relaxation task; following that they had one session of exposure to the odor paired with a stressful task and then were tested to observe whether the odor-stress conditioning occurred as measured by evaluation of autonomic arousal, cognitive function and self-reported stress and health symptoms. Data collection has been completed for this study and data analysis is largely complete.

AIM 3: PREVENTING THE ACQUISITION OF A LEARNED ASSOCIATION BETWEEN AN ODOR, STRESS AND SYMPTOMS.

If conditioned responses to previously neutral odors experienced in the context of a stressful event underlie the enhanced autonomic reactivity, stress and health symptom constellations that characterize GWS, then the literature on conditioning effects in animals and humans suggests that such associations can be prevented through mechanisms such as latent inhibition and blocking (Lubow & Moore, 1959; Kamin, 1968). Such mechanisms could be easily and usefully exploited to prepare troops prior to deployment in order to prevent the development of odor-stress associations that are likely to occur in combat or other military postings. The following experiments conducted under this aim will examine the characteristics of such preventive mechanisms.

Study 7: Latent Inhibition and Prevention of Odor-Stress Associations

Latent inhibition refers to the well-established principle of Pavlovian Conditioning (Lubow et al., 1959) whereby exposure to a CS (e.g., odor) before CS-US pairings can retard the acquisition of the conditioned response to the CS, relative to that of subjects who did not have CS pre-exposure. Two prior exposures to a CS (odor) were given. Group 1 was given exposure to the CS odor in a non-stressful (slideshow) condition and

Table 3. Design of Study 7

Group	Exposures		Test Session
1	CS_a+20 min.stressor	CS_a + 20 min. relaxation	CS_a - HR/Resp/Cog
2	CS_b+20 min. relaxation	CS_b + 20 min.stressor	CS_a - HR/Resp/Cog.

Subjects: Twenty four subjects were tested in each group, yielding a total of 48 subjects. The ethnic and gender breakdown are presented in Table 4. Average age of the subjects was 28.8 for the females, 27.6 for the males.

PROCEDURE: Subjects participated in three sessions, tested individually. The procedure for eliciting stress during the stress- conditioning session was identical to that described in the previous studies, with subjects exposed to an odor paired with a modified version of the Trier Social Stress Task (TSST). The TSST is a mental stress provocation task consisting of a 10 minute preparation/anticipation phase and a 10 minute performance-under-stress phase (Kirschbaum, Pirke, & Hellhammer, 1993). The subject was given 10 minutes to prepare a 5 minute oral speech which was recorded on videotape to be evaluated by a panel of judges. (Unlike in the real TSST, no evaluation actually took place). The instruction coincided with the dispersion of a detectable concentration of the Conditioning Odor. After 10 minutes of preparation, the experimenter announced the end of preparation and the start of the speech via intercom, and the videotape was started. No videos of the subject's performance were actually kept or judged and each was overwritten at the start of the next session (or erased, whichever was more feasible). Following the public speaking phase, the subject was switched to the mental arithmetic portion of the task, which required the subject to perform serial subtraction aloud.

Whenever necessary, the experimenter prompted the subject via intercom to increase their speed, to begin over (when a mistake was made), etc.

The procedure for eliciting latent inhibition involved exposing the individual to the to-be-conditioned odor in a non-stressful situation. Subjects were allowed to sit and watch a slideshow for 20 minutes while the odorant was dispersed into the chamber.

Subjects were alternately assigned to Group 1 or 2 depending on the week of test. Subjects assigned to Group 1 had the non-stressful condition first, followed by the stressful condition and then the test phase. Subjects assigned to Group 2 had the stressful condition first, followed by the non-stressful condition and then the test phase. Two days following the second exposure session, all were again exposed to the conditioning odor in the same room while the various endpoints were being measured, including self-reported stress, salivary cortisol, heart rate, respiration, health symptoms and memory measures.

Table 4. Enrollment of participants in Study 7

	Caucasian	**African/ American**	**Hispanic**	**Asian American**	**Other or Unknown**	**TOTAL**
Female	**11**	**9**	**2**	**1**	**0**	**23**
Male	**12**	**7**	**3**	**2**	**1**	**25**
TOTAL	**23**	**16**	**5**	**3**	**1**	**48**

Hypothesis: Our hypothesis was that the conditioned-stress response to the odor would be greater for those individuals who had the stress-odor pairing on the first session than for those individuals who had the stress-odor pairing on the second session. We also hypothesized that the control groups 3 & 4 (receiving the first two sessions without any odor) would not show the same level of enhanced stress responding on Session 3 as did either Group 1 or Group 2.

Data Analysis:

Using an omnibus MANOVA, we evaluated the main effect of session order (stress first vs. relaxation first) on the magnitude of the stress response in the TSST session and found no

significant overall effect of order, $F_{(1,47)} = 2.8$, $p > .1$ such that stress responses (HR, Cortisol, Self-reported stress) to the TSST manipulation was not affected by whether the TSST session occurred before or after the relaxing session. However, although initial stress responding did not look different, a MANOVA performed on the final test session showed that there was a main effect of session order, $F_{(1,,47)} = 11.6$, $p < .05$.

Subjective ratings of stress: There was a main effect of session order on perceived stress response in Session 3, $F_{1,47} = 4.95$, $p < .05$. Individuals who were exposed to the odor paired with the relaxation session first rated stress lower in Session 3 than did individuals who were exposed to the odor during the stressful session first (M=14.2 vs. 42.5). Stress ratings were made using a visual analogue scale and scored from 0-100.

Salivary Cortisol: Saliva samples were obtained at multiple timepoints during the third session in order to observe whether there were any stress-related increases in cortisol upon re-exposure to the odor during the test session. In contrast with the self-reported stress ratings, there were no significant differences between cortisol responses as a function of session order, however, there was a trend for a significant session by gender interaction, with females showing a heightened cortisol response on session 3 if they had been exposed to the odor paired with the stressor first, ($F_{1,47} = 4.36.$, $p = .08$). As noted in previous studies, both baseline cortisol levels and changes appear to be quite heterogenous, with some individuals showing large increases upon response to the stressor (and the subsequent re-exposure to the odor) while some individuals who nonethleless, report feeling stressed do not show much of an elevation in salivary cortisol. The elevation in cortisol response for some individuals could be considered clinically significant elevations in stress response, but overall the variance we observe suggests that salivary cortisol may not be the most reliable marker of stress across all individuals.

Performance on CVLT: Disruptions in cognitive performance, especially memory tasks, have historically been a salient symptom complaint from Gulf War veterans. To evaluate the role of odor-conditioned stress in eliciting cognitive impairments, we evaluated the degree to which conditioned stress could disrupt cognitive processing using the California Verbal Learning Test. We evaluated multiple dimensions of performance on the CVLT, including number correct on

free recall, number of repetitions and number of intrusions (items reported as being on the test which were not actually presented).

CVLT Results on Session 3

Group	Correct Recall	Repetitions	Intrusions
1 (Stress 1st)	7.11	1.8	3.95
2 (Relax 1st)	6.85	2.1	2.15

ANOVA analysis revealed that overall CVLT recall performance did not differ between the session order conditions for the two groups, $F_{(1,47)} = 1.58$, $p>.1$ ($M = 7.11.45, 6.85$). However, as before, we saw a significant interaction between NA and performance type (Recall, Repetitions, Intrusions) in which the group which had the stressor first exhibited significantly more intrusions when re-exposed to the stress associated odor than did the group who had the relaxer first, $F_{(1,47)} = 4.51$, $p <.05$; (mean difference in # of intrusions between conditions = 3.95, & 2.15, respectively). In addition, Group 1 self- reported poorer memory performance and greater effort on the test phase than did Group 2 (6.98 vs. 3.95 on a 10 point visual analog scale, respectively). This finding continues to be of interest given the reported claims among GW veterans of poorer memory performance, which are not always observed when tested using standard memory measures. In brief, this suggests that stress may interact with perceived effort such that cognitive processes are judged as more effortful and less efficient, even if the outcome on performance is the same. The increased perceived effort might have detrimental implications for information-processing under deployment situations.

Electrodermal response (EDR): To measure the degree of arousal orientation to the odor cue, skin conductance (EDR) was measured throughout each phase, although the analysis was confined to 1-minute epochs surrounding each of the timepoints where experimental manipulations occurred. Of greatest interest in this study was to observe whether the phasic EDR, which was time-locked to certain experimental manipulations, differed during session 3 as a function of extinction condition. Figure 1 presents the EDR response across the multiple time points of Session 3 expressed in microsiemens. Upon re-exposure to the odor in session 3, there was an increase in EDR for the group that had the odor paired with stress first (circles) compared with the group that had the odor paired with relaxation first (squares), but as in previous

experiments, this increase was not significant at the level of p = .008 (the level needed for Bonferroni correction for multiple comparisons).

The magnitude of any phasic response to a stimulus can range from 0.5 to 5 microsiemens. Thus, while the average amplitude of the phasic response, less than 1.5 microsiemens, is not very impressive, there does appear to be a mild orienting response to the introduction of the odor in the group that receive the odor paired with stress first. However, the amplitude of the EDR was higher at the start of the memory task, suggesting that the combination of odor + additional stress may have a greater impact than just exposure to the odor or the additional stress alone (note: no change in EDR for the group who had the relaxer first at the start of the CVLT). In general, EDR response should be considered merely an exploratory measure which can signify autonomic arousal, and tying the actual levels of the phasic response to a clinically significant stress level is problematic. Hence, at the present time, we look more toward the self-report and behavioral responses to indicate the presence of conditioned and extinguished stress response.

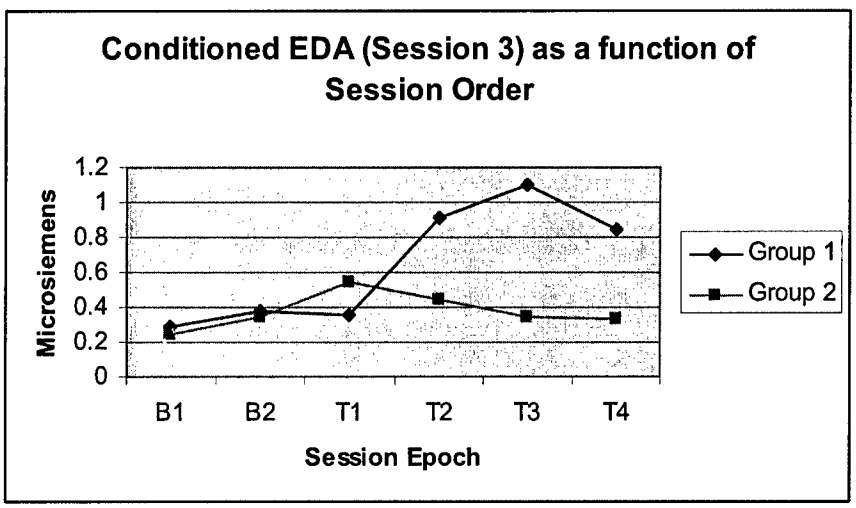

Figure 1: Electrodermal response as a function of session order. Circles represent the averaged response of the group that received the stressor first, Square symbols represent the group that received the relaxer first. Typical range of EDA is 0.5-5.0 microsiemens. B1= initial baseline in chamber, B2=10 min after entry into chamber, T1=introduction of odor into chamber & buildup (respirator on), T2=removal of respirator/odor cue, T3= start of CVLT, T4=end of exposure.

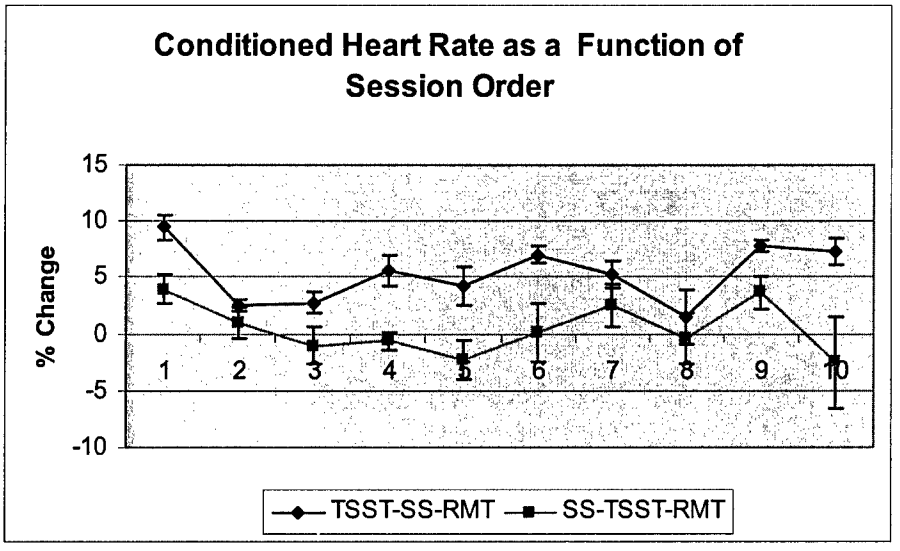

Figure 2. Heart rate during session 3 for group 1 (TSST first) and group 2 (SS first). The group experiencing the TSST paired with odor in the first session showed significantly more increased heart rate upon re-exposure to the odor on the third session.

Heart Rate: Heart rate was averaged into 10 epochs (2-min intervals during the 20-minute re-exposure to the odor in session 3). A repeated-measures ANOVA performed on the heart rate in session 3 revealed a nearly significant main effect of session order, $F_{1,47} = 15.5$, $p < 0.001$ ($p = .001$ needed after Bonferroni corrections). The average heart rates during session 3 mirrored the self-reported stress results and thus differed as a function of whether the group received the stress-odor pairing session first or second.

Reported Health Symptoms: Participants rated a variety of health symptoms immediately before and at the end of exposure on each session. 25 health symptoms were rated and for analysis, classified into 7 subgroups: Autonomic nervous system, gastrointestinal, central nervous system, cognitive, respiratory, irritation. To control for bias to respond, a number of control symptoms were also rated (i.e., leg cramps, tooth pain = sham condition).

There was a main effect of session order on health symptoms, $F_{(1,47)} = 8.21$, $p<.05.05$. However, a closer look at the symptom subgroups demonstrated that only several symptom clusters were responsible for the difference. There was a significant interaction between session order and health symptom groups, $F_{(6, 281)} = 5.56$, $p <.001$ (Bonferroni corrected p value of .007 for significance). Post hoc tests revealed that only the autonomic nervous system and cognitive

symptoms differed between groups, both at $p < .001$, with the group receiving the stressor session first having higher reports of symptoms on session 3. No other subgroups of symptoms differed between the groups.

Implications of Study 7 results: As was observed in earlier studies, the magnitude of the conditioned response in these studies is not large, especially when compared to what might be experienced in real-world settings. However, despite this limitation, we were able to demonstrate that the order in which an odor and stressor are experience can influence the magnitude of the conditioned response. This suggests that under controlled circumstances, prior exposure to the to-be experienced odor in a safe, non-stressful environment could possibly serve to reduce the formation of an association of that odor to stress and thus prevent the persistence of a stress-odor association in the future.

Study 8: Blocking and Prevention of Odor-Stress Associations

Study 8, entitled "Blocking and Prevention of Odor-Stress Associations", investigated the extent to which pre-associating a novel "scapegoat" odor with a stressor could neutralize the subsequent ability of other odors to become associated with stressors and prevent the acquisition of conditioned odor-stress responses. 36 out of a planned total of 40 subjects have been tested in three sessions in which they were exposed to either novel odors or more familiar ones, paired with stressors; the degree of odor-stress conditioning that occurred was measured by evaluation of autonomic arousal, cognitive function and self-reported stress and health symptoms.

Aim: Under many circumstances where odor-stress conditioning may be prophesized to occur, it may not be possible to identify the relevant, to-be-exposed odors prior to encounter in a stressful, field or combat situation. In this case, another mechanism would be required in order to prevent the formation of an associative response to the odor. The phenomenon of *blocking* refers to the well-established finding that presenting a target CS (e.g., Odor B) in the presence of another CS (Odor A) that has been previously paired with the same US (e.g., stress) interferes with the formation of an association by the target CS. Blocking has been shown to significantly reduce the formation of chemotherapy-related food aversions among patients who were exposed to a "scapegoat" food or beverage prior to their treatment (Mattes, 1994). We predict that if a novel odor has been previously associated with a stress response or autonomic arousal, then diesel fuel, petrochemical odor, or any odor that is subsequently experienced in the presence of that novel

odor will, in general, fail to become conditioned to the US stressor and elicit the conditioned response. Thus, pre-associating an odor to a stress response prior to deployment could effectively neutralize the potential of any other odor to become associated with a stressful response. The goal of Experiment 8 was to test this assumption.

Procedure: 36 subjects have been tested thus far and assigned to one of two groups. We are in the process of testing the final 4 in the upcoming weeks. The novel odor (osmanthus) was identified in a pilot study from a group of candidates that have previously been rated as "very unfamiliar" by American subjects. Group 1 had one session (blocking) in which they were exposed to an odor (CS_a - osmanthus) paired with the stressor task. One day later they returned for a second (conditioning) session in which they experienced both CS_a (osmanthus) and CS_b (balsam odor) during the stressor task. On Day 3 they returned for the test of conditioning, in which they were exposed to both the novel odor and balsam odor in counter-balanced order, while the autonomic and symptom endpoints were measured (as in Experiment 1). The control group had the same experience during Sessions 2 and 3, but during Session 1 (Blocking) they were exposed to a third novel odor (CS_c – leafy green) paired with the stressor task. This odor was not experienced again. On the second session, all subjects were told mechanical failure of the videorecorder necessitated repeating the stressor task (as was done in Study 2.)

Table 5. Design of Study 8

Group	Blocking Session	Conditioning Session	Test Session
1 (Blocking)	CS_a + Stressor	CS_a, CS_b + Stressor	CS_a - HR/Resp /Cog. CS_b -HR/Resp /Cog
2 (Control)	CS_c + Stressor	CS_a, CS_b + Stressor	CS_a - HR/Resp/ Cog. CS_b -HR/Resp /Cog

Statistical Analysis: The same endpoints were measured and analyzed as in previous studies.
Hypothesis: We hypothesized that subjects in both groups would show some conditioning of autonomic stress responses and health symptoms to CS_a, but that subjects in Group 1 would show less conditioning of autonomic responses, health symptoms and cognitive impairment to balsam odor/CS_b than would subjects in Group 2. This would occur because the acquisition of

the association between balsam and stress when CS_a was present was "blocked" by the prior association between CS_a and stress.

Table 6. Enrollment to date in Study 8

	Caucasian	African/ American	Hispanic	Asian American	Other or Unknown	TOTAL
Female	**11**	**7**	**1**	**0**	**0**	**19**
Male	**12**	**5**	**0**	**0**	**0**	**17**
TOTAL	**23**	**12**	**1**	**0**	**0**	**36**

Data from Session 3 were analyzed using Analysis of Variance (ANOVA).

Subjective ratings of stress: There was a main effect of group on perceived stress response, $F_{(2, 35)} = 4.17$, $p < .05$, with subjects in the blocked group reporting less stress during session 3 than subjects in the unblocked group.

Salivary Cortisol: As data collection in this study is not yet complete, we have not yet submitted the salivary samples to the analytical lab (for consistency reasons, it is important that all to-be-compared samples are run with the same set of internal controls). Thus, the results of the cortisol analysis are still pending.

Performance on CVLT: As in prior studies, we evaluated multiple dimensions of performance on the CVLT, including number correct on free recall, number of repetitions and number of intrusions (items reported as being on the test which were not actually presented).

Group	Correct Recall	Repetitions	Intrusions
Blocked	8.35	.55	2.1

Unblocked	7.95	3.8	4.2

Analysis of variance performed on the free recall, repetitions and intrusions revealed a main effect of group (blocking condition), $F_{(1, 35)} = 5.12$, $p < .05$. We also observed a significant interaction between group and performance type (recall, repetition, intrusion), $F_{(4, 70)} = 3.85$, $p < .05$. The'blocked' group had fewer repetitions than the 'unblocked' group, Free recall performance during re-exposure to the odorant in session 3 did not differ as a function of blocking condition, but the number of intrusions did, with more intrusions (words generated which were not actually presented during the learning phase) in the group which did not receive 'blocking' than in the group that did. The group that did not receive the 'blocking' manipulation also reported poorer memory performance on the test phase than did the other group (4.42 vs. 6.63 on a 10 point visual analog scale, but this did not reach significance.

Electrodermal response: Skin conductance was measured throughout Session 3, although the analysis was always confined to averages during 1-minute epochs surrounding each of the timepoints where experimental manipulations occurred. The main comparison we have completed thus far is to observe whether the phasic skin conductance response to the reintroduction of the odorant during session 3 differed as a function of group. There was a non-significant effect of Group, $F_{(1,35)} = 3.52$, $p = .05$ (Bonferroni corrected p value of .008), but as reported previously, this measure is notoriously variable and thus may not confer the best indication of the strength of the conditioned effect.

Heart Rate: The percent change in heart rate from baseline to re-exposure during session 3 did, however differ as a function of group, with Group 1, having the but the main effect of condition was only marginally significant $F_{(1,35)} = 6.93$, $p = .01$, when corrected for multiple comparisons ($p = .008$). It is possible that this measure

Reported Health Symptoms: Participants rated a list of health symptoms immediately before and at the end of exposure on all days, using a 0-5 point scale in which 0 was used to signify they were not experiencing that symptom while 5 indicated they were experiencing the symptom to a great degree. As before, 25 health symptoms were rated and for analysis, they were classified into 7 subgroups: autonomic nervous system (ANS) gastrointestinal (GI), central nervous system (CNS), cognitive (COG), respiratory, irritation (RI). To control for bias to respond, a number of control symptoms were also rated (i.e., leg cramps, tooth pain = sham condition).

Analysis of symptom reports on Session 3 showed that there was an overall effect of group, $F(1,35) = 8.53$, $p < .05$, with the 'blocked' group reporting fewer and less intense symptoms overall than the 'unblocked' group. Closer inspection revealed that the significant effect was due to symptom reports in only three subgroups: ANS, CNS and COG. Control symptoms did not differ between the two groups.

Implications of Study 8: If the relevant odors to which soldiers might be exposed during deployment cannot be identified in advance, a training scenario in which a "scapegoat" odor (one not likely to be encountered during normal living conditions) is experienced during stressful training, could be utilized. This 'scapegoat' odor could be used during actual deployment to "block" the formation of additional odor-stress responses.

Overall Implications: The goal of studies during this period was to evaluate in the laboratory, the ability to prevent the development of odor-stress conditioned responses. The results of the studies thus far, although marginal, appear to hold some promise.

To evaluate whether the results observed in the current studies represent clinically significant reductions in the levels of stress is a difficult endeavor. To be sure, there are some consistent responses under stress conditioning and re-exposure that would seem to indicate a reliable production of a stress response to an odor cue. However, as we have observed in the past, the correlation between cortisol increase (or any other psychophysiological measure) and self-perceived stress or performance disruptions on memory test are inconsistent across subjects, suggesting that even in the presence/absence of a hormone response an individual may experience stress and this stress may interfere with performance and ultimately lead to health problems. The failure to find strong correlations between objective and subjective measures of stress and performance measures is not unique to our studies (e.g., (Karkow et al., 2004), but suggests that other variables which are not currently being measured may contribute to one or more measures of the stress response. To this end, in the study to be conducted during the one-year extension (on more ecologically relevant stress induction) we will obtain measures of chronic stress, as well as evaluate factors that may contribute to the stress response in general, such as sleeplessness, exhaustion, degree of social support, etc. (Dahlgren, Akerstedt, & Kecklund, 2004; Rosal, King, Ma, & Reed, 2004).

HUMAN SUBJECT PROTECTIONS

In response to initial concerns raised by the Institutional Review Board of the University of Pennsylvania and the Human Use Committee of the Army, we have taken additional measures to ensure the protection of subject participants in the studies described herein. In particular, one concern was expressed regarding the potential of the study manipulations introducing into participants an ongoing aversion to certain odors.

We acknowledged upfront that such a potential is present. To preclude this possibility, we continue to employ odors that are uncommon and dissimilar to odors generally experienced in the environment. Odors that we employ are a blend of *hinoki* and *galbanum*, *leafy green* or *osmanthus*, odors, which are familiar in other cultures (Japan) but are very unfamiliar to the western world. Another odor we employ is a fragrance blend crafted for us using primarily Asian floral ingredients, which is also rated as very unfamiliar by our participants. To date we have had no post-experimental complaints or reports from participants alleging any persistent aftereffects from this study. No allergic responses to the fragrances were anticipated and none have been observed or reported.

To date, no adverse events (either minor or serious) have been reported to us at anytime. We have had several withdrawals from the study, but these were due to conflicts with scheduling sessions, not, to our knowledge, due to any adverse reaction experienced by any subject. In order to minimize the potential for such effects, we have used laboratory stressors which are rather benign (public speaking and mental arithmetic). While increased stress levels (via self-report and physiological changes) have been noted among many of the participants following the stress manipulation, such effects have appeared fairly transient. In fact, the need to minimize the impact on subjects may to some extent compromise our ability to view the impact of odor-stress conditioning at any level close to what might be experienced in a real-world setting.

Important to our need to use deception for this study is that subjects *will* consent to some potentially stressful and unpleasant experiences in advance, without knowing exactly the nature of those experiences (which would serve to neutralize the stress value). Of course, we make certain the subject understands on multiple occasions that they can withdraw from the study at any time without penalty.

To ensure safety and prompt reactions in response to participant distress during the experimental procedure, 1) participants will be continuously monitored through video surveillance by the investigator, and 2) heart rate and respiration frequency, which are monitored during the entire experiment, will be visible to the investigator on a computer display outside of the testing chamber.

All subjects receive a formal **debriefing** following the last session, in addition to their ability to ask and have answered any questions regarding the study or their reactions. The debriefing addresses the purposes of the experiment as well as issues such as the possibility of carry-over to real life. Together with the consent form, these documents provide participants with an opportunity to contact us or the Institutional Review Board in case of questions or when experiencing side-effects from participation in our study. As stated earlier, no such reports have been made.

KEY RESEARCH ACCOMPLISHMENTS:

- Observed that pairing the odor with a relaxing experience prior to associating it with a stressful experience significantly reduced the ability of the odorant itself to elicit a stress response on subsequent re-exposure. This outcome has relevance for preventing odor-stress associations for individuals who might be deployed in novel odiferous settings where stress and other adverse experiences are likely.
- Observed that if a stressor is pre-conditioned to an odor that is not again encountered, this can reduce the possibility that another novel odor can be associated with the stress response.

REPORTABLE OUTCOMES:

1 presentation was made at the Association of Chemoreception Sciences Meeting in Sarasota, Florida in April 2005 reporting the results of Study 6.

A presentation was made at an NSF sponsored conference in Arlington, VA in August, 2005, reporting the results of Studies 6 and 7.

Several manuscripts are in preparation reporting the results of Study 6 and 7.

CONCLUSIONS:

Odors that are paired with a stressful situation appear to subsequently elicit a negative response that is not observed to the same degree when only the experimental context is present during conditioning and test phases. This response can be seen in self-reported annoyance to the odor, self-reported stress ratings during odor exposure, and judged, but not objective, performance on a cognitive learning and memory task. Perhaps most importantly, there is suggestive evidence that this association can be inhibited if the target odor or odors (not investigate) can be experienced in a non-stressful context prior to deployment. In addition, if the target odor or odors cannot be identified, there is additional evidence that associating a scape-goat odor to a stressful state which is likely to be experienced in deployment situations could aid to 'block' the formation of a conditioned response to the odors. Although the stressors used in this study, and their apparent effects on autonomic nervous system parameters are orders of magnitude weaker than those which will be experienced during deployment situations, the paradigm investigated in this project appears to be a useful laboratory-based model system for examining and understanding the persistent symptom constellations found in GWS and other stress-mediated syndromes.

REFERENCES:

References

Binder, L. M., Storzbach, D., Kent Anger, W., Campbell, K. A., Rohlman, D. S., & Other Members of the Portland Environmental Hazards Research Center (1999). Subjective cognitive complaints, affective distress, and objective cognitive performance in Persian Gulf War veterans. *Archives of Clinical Neuropsychology, 14,* 531-536.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24,* 385-396.

Dalton, P. (1996). Odor perception and beliefs about risk. *Chemical Senses, 21,* 447-458.

Dalton, P., Gould, M., Girten, B., Stodieck, L. S., & Bateman, T. A. (2003). Preventing annoyance from odors in spaceflight: a method for evaluating the sensory impact of rodent housing. *J.Appl.Physiol, 95,* 2113-2121.

Dalton, P., Wysocki, C. J., Brody, M. J., & Lawley, H. J. (1997). The influence of cognitive bias on the perceived odor, irritation and health symptoms from chemical exposure. *International Archives of Occupational & Environmental Health, 69,* 407-417.

Green, B. G., Dalton, P., Cowart, B. J., Shaffer, G., Rankin, K. R., & Higgins, J. (1996). Evaluating the "Labeled Magnitude Scale" for measuring sensations of taste and smell. *Chemical Senses, 21,* 323-334.

Kamin, L. J. (1968). "Attention-like" processes in classical conditioning. In M.R.Jones (Ed.), *Miami Symposium on the Prediction of Behavior: Aversive Stimulation* (pp. 9-31). Miami: University of Miami.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'. A Tool for Investigating Psychobiological Stress Responses in a Laboratory Setting. *Neuropsychobiology, 28,* 76-81.

Lees, P. S. J., Stefaniak, A., Emmett, E. A., & Dalton, P. (2003). Exposure assessment for study of olfactory function in workers exposed to styrene in the reinforced-plastic industry. *American Journal of Industrial Medicine, 44,* 12-23.

Lubow, R. E. & Moore, A. V. (1959). Latent inhibition: The effect of nonreinforced exposure to the conditioned stimulus. *Journal of Comparative and Physiological Psychology, 52,* 415-419.

Mattes, R. D. (1994). Prevention of food aversions in cancer patients during treatment. *Nutrition and Cancer, 21,* 13-24.

McNair, D. M., Lorr, M., & Droppleman, L. F. (1992). *Manual: Profile of Mood States. Revised.* San Diego: Education and Industrial Testing Service.

Smeets, M. A., Maute, C. M., & Dalton, P. (2002). Acute sensory irritation from exposure to isopropanol in workers and controls: Objective versus subjective effects. *Annals of Occupational Hygiene,* 359-373.

APPENDICES: None